Effects of age at seroconversion and baseline HIV RNA level on the loss of CD4+ cells among persons with hemophilia

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Objective: To assess the impact of age at seroconversion and HIV RNA level in serum during early chronic infection on the initial values and subsequent trends (slopes) of CD4+ lymphocyte counts.

Design and methods: In a cohort of 137 HIV-1-positive hemophiliacs with well-estimated dates of seroconversion, baseline HIV RNA level was measured by reverse transcription PCR in serum specimens collected 12–36 months after the estimated date of seroconversion. Baseline values, 24 months after seroconversion, and slopes of CD4+ lymphocyte counts by age and HIV RNA quartile were examined by fitting random effects models that allowed for intrasubject variability.

Results: Both age at seroconversion and HIV RNA level were associated with the CD4+ lymphocyte count at baseline and its subsequent slope. The baseline median CD4+ lymphocyte count was 620×10^6/l. Within each HIV RNA quartile, the median CD4+ cell count of the oldest subjects (age 30–58 years) was about 200×10^6/l lower and at least 350×10^6/l lower than the median counts of the younger (age 11–29 years) and youngest (age 2–10 years) subjects, respectively. Within each age-group, the median CD4+ cell count differed by about 200×10^6/l between subjects in the lowest compared with the highest HIV RNA quartiles. The mean slope of the CD4+ lymphocyte count after month 24 was linear on the square-root scale, steeper in children, and did not vary significantly by baseline HIV RNA quartile. There was large variation between subjects that was unexplained by differences in age and HIV RNA level.

Conclusions: By 24 months after HIV seroconversion, the oldest subjects and those with the highest HIV RNA levels during early chronic infection had experienced the most severe depletion of CD4+ cells. Subsequent declines in CD4+ cells varied little by early chronic HIV RNA level or age.

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Keywords: Age, HIV RNA, CD4 cell counts, hemophilia, longitudinal studies, PCR, statistical linear models

Introduction

Declining numbers of CD4+ cells is a hallmark of infection with HIV, but the rate of decline varies substantially among individuals [1–7]. It is well established that older age at the time of HIV antibody seroconversion is associated with shorter times to AIDS [8–14]. However, the relationship of age to trends in the CD4+ cell count remains unsettled [5,6,14].

It now is possible to quantify the amount of virus (HIV RNA level) circulating in the blood. This advance has

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allowed the relationship between virus level and subsequent disease risk to be evaluated. The serum or plasma level of HIV RNA is high during primary HIV infection, but it falls to a lower, relatively constant level during chronic infection [15–17]. There are, however, large differences among subjects in the levels of circulating virus in plasma during early chronic infection [15,16,18]. Recent studies have shown that the HIV RNA level during early chronic infection is a strong predictor of disease progression [16,18–20]. Furthermore, higher HIV RNA levels are associated with lower numbers of CD4+ cells [17,19,21,22] and with a steeper decline in CD4+ cell counts in follow-up of prevalent cohorts [23,24]. However, the relationship between the HIV RNA level during early chronic infection and trends in the CD4+ cell count during the entire AIDS incubation period has not been fully investigated.

In this study, we assess whether age at seroconversion and the HIV RNA level in serum during early chronic infection (12–36 months after seroconversion) are significantly and independently associated with the trend in the CD4+ cell count. We investigated these associations in more than 10 years of follow-up of a subset of subjects in the Multicenter Hemophilia Cohort Study (MHCS).

Methods

Patients

Subjects were enrolled in the MHCS, an ongoing cohort study of persons with hemophilia that was initiated in 1982. Details on the MHCS are presented elsewhere [9,18]. For each HIV-1-infected subject, the date of seroconversion was estimated using each subject's blood product history and a statistical reconstruction of the incidence of infections in the cohort [25]. Most of the 137 subjects in the current analysis were 'observed seroconverters', as 119 (87%) had a 'last negative' serum specimen. Clinical information and data on laboratory parameters were collected every 6–12 months. However, for the majority of the subjects, the CD4+ cell count was routinely measured starting at least 2 years after seroconversion. The median time of the initial CD4 cell measurement was 3.5 years after seroconversion.

The analysis included all patients at five US hemophilia treatment centers who had a frozen (−70°C) serum specimen collected 12–36 months after the estimated date of seroconversion [18]. HIV RNA levels were measured using the HIV Amplicor Monitor assay (Roche Diagnostics, Nutley, New Jersey, USA). HIV RNA levels from specimens collected before 1983 were excluded because these specimens gave significantly lower values than later specimens, probably because they had not been collected, handled, and stored in compliance with a uniform protocol. CD4+ cell proportion and counts were determined at the study site or by a central laboratory using standard methods [9,18]. Because the slope of the CD4+ cell count was the primary endpoint of interest, only patients who had at least two CD4+ cell counts taken during follow-up were included in the analysis. CD4 cell data were censored at the date of the earliest opportunistic infection or malignancy that met the 1987 AIDS surveillance definition of the US Centers for Disease Control and Prevention.

Statistical analysis

Two random effects models (see Appendix) were used to assess whether age and HIV RNA level during early chronic infection affect the population slope or baseline level of CD4+ cell count. The first allowed a different baseline count for the youngest group [26,27]. The second allowed a different baseline count for each of three age-groups (35 aged ≤ 10 years, 69 aged 11–29 years, 33 aged ≥ 30 years) and for each HIV RNA quartile (28 with < 2000 copies/ml, 40 with 2000–4999 copies/ml, 40 with 5000–19 999 copies/ml, 29 with ≥ 20 000 copies/ml). The cut-off points for age were based on its relationship with CD4+ cell count [26,27] and the AIDS incubation distribution [9]. We assessed departures from linearity by fitting higher order terms as population mean parameters, and evaluated model fit using the log-likelihood ratio test. Restricted maximum likelihood methods were applied to estimate the model parameters [28,29]. Normality assumptions were checked by the normal score plots. Sensitivity analyses (not presented in detail) were performed through the use of ordinary least squares unweighted models and by examining random effects models on subgroups with at least three or at least five CD4 cell values. For presentation of results, mean CD4+ lymphocyte counts were rounded to the nearest 10×10^6/l and HIV RNA levels to the nearest 100 copies/ml.

Results

Study subjects

Of 165 individuals in the MHCS with HIV-1 RNA levels measured during early chronic infection, 137 (83%) subjects with at least two CD4+ cell counts taken during follow-up were included in the analysis. The 28 excluded individuals progressed to AIDS more rapidly than those included in the analysis ($P < 0.001$) and had higher mean HIV RNA levels ($P = 0.002$). Selected baseline characteristics of the study population are given in Table 1. The majority of the 137 subjects had severe type A hemophilia (factor VIII deficiency). Their mean seroconversion age was 19.5 years (range,

Table 1. Descriptive characteristics of 137 persons with hemophilia, HIV RNA measurements and at least two CD4+ cell count determinations.

	Values
Coagulation disorder [n (%)]	
Hemophilia A	122 (89.1)
Hemophilia B	14 (10.2)
Other	1 (0.7)
Severity of coagulopathy [n (%)]	
Mild	11 (8.0)
Moderate	11 (8.0)
Severe	115 (84.0)
Mean (SD) age (years)	19.5 (13.3)
Mean (SD) HIV RNA ($\log_{10}$ copies/ml)	
12–36 months after HIV seroconversion	3.7 (0.8)
Median (interquartile range) time of first CD4+ T-cell measurement after HIV seroconversion (years)	3.5 (2.0–4.6)

2.4–58 years). HIV RNA levels ranged from less than 200 copies/ml (n = 4) to 237 300 copies/ml. With the four undetectable HIV RNA levels arbitrarily set to 100 copies/ml, the geometric mean HIV RNA level was 5400 copies/ml [95% confidence interval (CI), 200–183 600].

The median time from seroconversion to last CD4 cell evaluation was 10 years (range, 7.7–11.4 years). On average, nine CD4+ cell counts per subject were taken with the median time being 0.6 years between any two adjacent measurements. By the end of follow-up, 41 subjects had developed AIDS and 13 had died without a diagnosis of AIDS.

Effects of age and HIV RNA level on the population mean trend in CD4+ cell counts

The estimated median CD4+ cell count at baseline (2 years after seroconversion) was 620×10^6/l (95% CI, $560–680 \times 10^6$/l). The population mean CD4+ cell count slope, beyond 2 years after seroconversion, was fairly linear on the square-root scale, with a mean slope

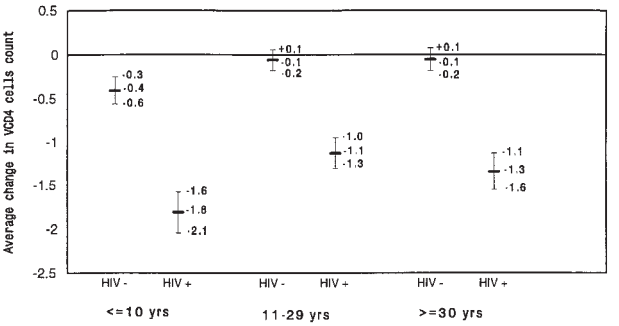

Fig. 1. Estimated mean change (mean slope, ± 95% confidence interval) in CD4+ cell count on the square-root scale by age and HIV infection status using a random effects model that allowed for a different baseline CD4+ cell count in the youngest age-group. The HIV-negative subjects with hemophilia were matched to the HIV-positive subjects on exact year of age at the time of the seroconversion event.

(change in $\sqrt{CD4}$ cell count) of −1.42 per year (95% CI, −1.18 to −1.66).

To assess the effect of age at seroconversion and HIV RNA level on the population mean CD4 cell slope, we fitted a random effects model that allowed the population mean slope to vary across age and HIV RNA groups. In this model, children (≤ 10 years) had a separate population mean baseline CD4+ cell count. The mean CD4 cell slope differed significantly by both age ($P = 0.004$) and HIV RNA level ($P = 0.04$; Fig. 1). Within each HIV RNA level, CD4 cell slopes were steepest (most negative) in the youngest age-group, but part of this decline could be due to natural loss of CD4+ cells during childhood. Amongst adults, CD4+ slopes were steeper for subjects older than 30 years. Within each age-group, there was a monotonic relation between HIV RNA level and CD4 cell slopes, with steeper slopes at higher levels of HIV RNA. Slopes

Table 2. Effects of age at HIV seroconversion and baseline HIV RNA level on square-root transformed CD4+ cell count*.

	Age at seroconversion (years)			
	≤ 10	11–29	≥ 30	All subjects
Mean (SD) baseline square-root CD4 cell count				
Baseline HIV RNA level (copies/ml)				
< 2000	31.2 (1.6)	27.8 (1.3)	23.8 (1.6)	27.5 (1.3)
2000–4999	29.0 (1.5)	25.5 (1.1)	21.5 (1.5)	25.4 (1.1)
5000–19999	27.0 (1.3)	23.6 (1.2)	19.6 (1.3)	23.4 (1.1)
≥ 20000	27.2 (1.4)	23.8 (1.5)	19.8 (1.6)	24.0 (1.3)
All subjects	28.0 (1.1)	25.3 (0.8)	20.7 (1.2)	24.9 (0.6)
Mean (SD) slope of square-root CD4 cell count				
Baseline HIV RNA level (copies/ml)				
< 2000	–1.89 (0.34)	–1.35 (0.25)	–1.27 (0.34)	–1.43 (0.26)
2000–4999	–1.71 (0.31)	–1.17 (0.24)	–1.09 (0.33)	–1.26 (0.23)
5000–19999	–1.83 (0.29)	–1.30 (0.26)	–1.21 (0.31)	–1.42 (0.22)
≥ 20000	–2.03 (0.31)	–1.50 (0.33)	–1.41 (0.37)	–1.72 (0.29)
All subjects	–1.87 (0.22)	–1.28 (0.17)	–1.21 (0.26)	–1.42 (0.12)

*The random effects model, with different baseline CD4+ cell counts and slopes by age-group and HIV RNA quartile, had the following subject-specific effects: SD of baseline count, 5.43; SD of slope, 1.16; correlation between baseline count and slope, –0.57. The within-subject SD was 3.56.

Table 3. Median CD4+ lymphocyte count ($\times 10^6$/l) at 2, 6 and 10 years after HIV-1 seroconversion.

	Seroconversion age [median (95% CI)]		
	≤ 10 years	11–29 years	≥ 30 years
Year 2 after seroconversion			
Baseline HIV RNA level (copies/ml)			
< 2000	980 (790–1180)	770 (640–920)	570 (430–720)
2000–4999	840 (680–1010)	650 (540–770)	460 (340–600)
5000–19999	730 (600–880)	556 (450–670)	380 (290–490)
≥ 20000	740 (600–900)	564 (440–700)	390 (280–520)
Year 6 after seroconversion			
Baseline HIV RNA level (copies/ml)			
< 2000	560 (450–680)	500 (420–590)	350 (270–450)
2000–4999	500 (390–600)	433 (370–510)	290 (220–380)
5000–19999	390 (310–470)	338 (270–410)	220 (160–290)
≥ 20000	360 (290–450)	316 (240–400)	200 (140–270)
Year 10 after seroconversion			
Baseline HIV RNA level (copies/ml)			
< 2000	260 (150–410)	290 (190–410)	190 (90–310)
2000–4999	230 (130–360)	260 (180–360)	160 (80–280)
5000–19999	160 (80–250)	170 (100–270)	100 (40–190)
≥ 20000	120 (50–220)	140 (60–240)	70 (10–160)

were 1.5–1.9-fold steeper for the highest compared with the lowest HIV RNA quartile (Fig. 2).

In a subsequent model (Table 2), we also allowed the population mean baseline CD4+ cell count to vary by age and HIV RNA groups. Both age and HIV RNA were significantly related to the mean baseline CD4+ cell count ($P < 0.001$), with higher counts among younger subjects and those with lower levels of serum HIV RNA. In contrast, CD4 cell slopes were not significantly related to HIV RNA level, did not differ between the two adult groups, and were only slightly steeper among children compared with adults ($P > 0.20$). The negative correlation between baseline CD4+ cell count and slope (–0.57; Table 2) indicates that a higher baseline was associated with a steeper decline.

The estimated median CD4+ cell counts at 2, 6 and 10 years after seroconversion, according to this final model, are shown in Table 3. Median CD4+ cell counts at 2 years after seroconversion differed between the youngest and the oldest age-group by more than 350×10^6/l. The two adult groups differed by about 200×10^6/l. Within each age-group, CD4+ cell counts differed by about 200×10^6/l between the highest and lowest HIV RNA quartile. Although the trajectories of the median CD4+ cell count by age-group and HIV RNA level became closer over time, there was a substantial difference among groups at 6 and 10 years after seroconversion. Six years after seroconversion, the median CD4+ cell count differed between the youngest and the oldest age-groups by more than 150×10^6/l, and by more than 120×10^6/l between highest and lowest HIV RNA quartiles. There was no evidence of multiplicative statistical interactions between age at seroconversion and HIV RNA level on the levels of CD4+ cells ($P > 0.20$), but this study may have had insufficient subjects to detect such interactions.

Between- and within-subject variability

In the final model, age and HIV RNA level explained 25% of the between-subject variability in the baseline CD4+ cell count and only 7% of the between-subject variability in slope. The remaining variability was large, as reflected by the between-subject SD of the baseline CD4+ cell count and slope (5.43 and 1.16, respectively; Table 2). Within-subject variability also was substantial (SD, 3.56; Table 2). On the square-root scale, this implies that the variation in the CD4+ cell count decreased with the underlying count. For an underlying CD4+ cell count of 500, 200 and 100×10^6/l, the estimated SD were 159, 101 and 71,

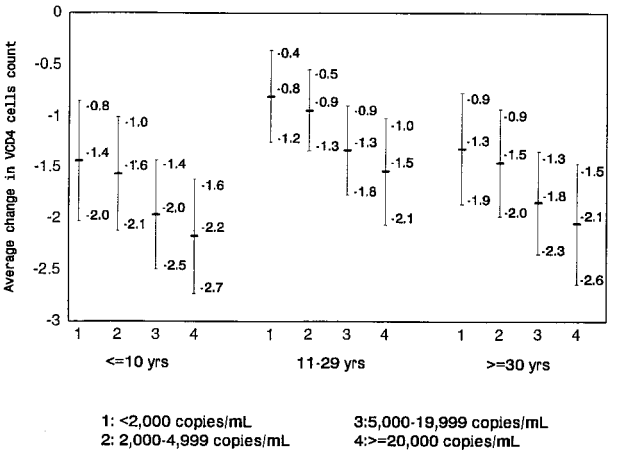

Fig. 2. Estimated mean change (mean slope, ± 95% confidence interval) in CD4+ cell count on the square-root scale by age and HIV RNA group using a random effects model that allowed for a different baseline CD4+ cell count in the youngest age-group.

respectively. Thus, a substantial part of the total variation in the CD4+ cell count is due to biologic variation or measurement error.

Discussion

In our cohort of 137 HIV-positive hemophiliacs with well-estimated seroconversion dates, the population mean CD4+ cell count declined linearly on the square-root scale subsequent to the first 2 years after seroconversion. This implied that the loss of total CD4+ cells gradually slows over time. This finding was qualitatively consistent with the results from four other cohort studies [3–7,30,31]. Quantitatively, the estimated mean CD4+ drop per year (1.42 on the square-root scale) was slightly lower than the declines reported for other cohorts, perhaps because HIV RNA levels were unavailable for some subjects who rapidly progressed to AIDS.

Amongst uninfected individuals, absolute counts of all T-cell subsets decrease from birth to age 10 years and then remain relatively stable. This pattern also holds for people with various clotting disorders but without lymphocyte abnormalities [26,27]. In the current study of HIV-infected hemophiliacs, younger age was associated with higher number of CD4+ cells at 2 years after seroconversion. Compared with older subjects, the CD4 cell slope more than 2 years after seroconversion was steeper in children aged 0–10 years, but part of this decline was due to the children's normal CD4+ cell loss and should not be attributed entirely to HIV infection [14,26,27]. The estimated population mean CD4+ cell trajectory for children should be interpreted cautiously, since the magnitude as well as the duration of the normal CD4+ cell loss depends on the exact age of each child. When we allowed the mean baseline CD4+ cell count to vary by age-group, the mean rate of CD4+ cell count decline did not differ significantly between the two adult age-groups.

It has been established that younger age at seroconversion is associated with slower progression to AIDS [8–10,13], but several studies have failed to show an effect of age at seroconversion on trends in the CD4+ cell count [5,6,14]. Results from the Multicenter AIDS Cohort Study (MACS) indicated a faster mean rate of CD4+ cell decline in older subjects, although this finding was not statistically significant at the 5% level [6]. In a previous analysis of our hemophilia cohort [9], we found a significantly higher rate of low CD4+ cell counts after seroconversion among older adults than in younger subjects. Our analysis suggests that the more rapid depletion of CD4+ cells amongst older adults occurs primarily during the first 2 years after HIV seroconversion.

Recent studies have reported that high HIV RNA levels during early chronic infection predict more rapid development of AIDS even after controlling for age and current CD4+ cell count [16,18–20]. It has also been reported that the number of circulating HIV RNA copies was inversely related to CD4+ cell count [17,21,32] and that increases in CD4+ cell count during antiviral drug therapy corresponded to decreases in viral load [33–35]. Mellors *et al.* [23] found that the higher the HIV RNA concentrations at baseline (study entry) the greater the rate of subsequent decline in CD4+ cell count. Likewise, Hughes *et al.* [24], in data from a randomized clinical trial, found that the mean change in CD4+ cells at week 48 (time starting at randomization) was significantly associated with the plasma HIV RNA level at baseline. In our study, when we allowed population mean CD4+ cell count decline to vary by HIV RNA level, the results were qualitatively and quantitatively similar to those from MACS [23]. However, when we also allowed the mean baseline CD4+ cell count to vary across HIV RNA level, we found that the levels of serum HIV RNA 12–36 months after HIV seroconversion were significantly associated with the degree of CD4+ depletion by the second year after seroconversion, but there was little evidence that HIV RNA levels during early chronic infection had a continued effect on the rate of CD4+ cell decline. Possible biases, listed below, or the relatively small size of our study may obscure the relationship of baseline HIV RNA levels with the subsequent rate of CD4+ cell count decline during the entire AIDS incubation period. However, our findings point out the importance of early HIV virologic–immunologic events on the rate of progression to AIDS. Our results indicate that subjects with higher levels of HIV RNA early in the course of infection are likely to reach a critically low CD4+ cell count sooner than subjects with lower levels, and are consistent with HIV RNA being an early and strong prognostic factor of disease progression.

Selection bias may have affected our results. Of the 165 subjects with measured HIV RNA levels during early chronic infection, 28 (17%) were excluded from our analysis because they had less than two CD4+ cell measurements. It is likely that the CD4+ cell decline was steeper for these patients than for patients included in the analysis. The exclusion of these patients is likely to cause an underestimate in the mean rate of CD4+ cell decline and, perhaps, in the effects of age at seroconversion and HIV RNA levels on mean CD4+ trends. Another possible source of bias with a similar effect is informative censoring. Specifically, subjects who progress to AIDS tend to have fewer CD4 cell measurements and thus less stable subject-specific estimates than those who do not progress. If the progressing subjects also tend to have steeper rates of CD4+ cell decline, then the mean rate of CD4+ loss estimated by

random effects models, which is a weighted mean of the subject-specific estimates with weights proportional to their precision, will also be biased downwards. However, sensitivity analysis limited to subjects with at least three CD4 cell values, at least five CD4 cell values, and using unweighted means gave similar results (not shown). Single agent antiretroviral therapy, which was received by some subjects during the study period, could be another source of bias. Such treatment, however, has only a small and transient effect on CD4+ cell trends, and models that ignore this effect fit the data almost as well [36].

Although the CD4+ cell count trajectories for the population are estimated well, as indicated by the relative small SD, individual trends may well depart from the means. This is shown by the remaining large between- and within-subject variability, especially at higher CD4+ cell counts.

In conclusion, the population mean CD4+ cell decline more than 2 years after seroconversion is approximately linear on the square-root scale. Both age at seroconversion and HIV RNA level early in the course of infection are significant and independent determinants of the CD4 cell trends, mainly of the degree of the CD4+ cell depletion during the first 2 years after seroconversion. Age at seroconversion and early HIV RNA level did not appear to have a continued effect on an individual's rate of CD4 cell decline. Furthermore, these factors are not the sole determinants of individual trends, as shown by the remaining large between-subject variability.

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Appendix

For the statistical analysis, we square-root-transformed CD4+ cell counts to reduce skewness and to linearize changes over time. Rather than linear regression, which loses validity when the number of observations (CD4 cell values) is not the same for all subjects, we fitted random effects models to characterize the trends in $\sqrt{CD4}$ cell counts [28,37–39]. In these random effects models, the CD4+ cell count is partitioned into the sum of three components, the population mean, a subject-specific effect, and a residual. The general form of the model is as follows:

$$\sqrt{CD4}_{ij} = \mathbf{X}_{ij}\alpha + \mathbf{Z}_{ij}\beta_i + e_{ij}.$$

In this expression, $CD4_{ij}$ is the CD4+ cell count for subject i at time t_j, $\mathbf{X}_{ij}$ is a p-vector of population-level covariates with population mean regression parameter α (e.g., overall or age-specific intercept and slope), $\mathbf{Z}_{ij}$ is a q-vector of subject-specific factors, β_i are subject-specific random coefficients that measure the deviation of the individual trajectory from the mean for the population to which the individual belongs, and e_{ij} is the within-subject residual or 'error'.

Random effects models are designed to 'borrow strength' across subjects when data are sparse, in order to estimate each subject's parameters β_i. They do so by assuming that the β_i are independent and identically distributed normal variates, with mean 0 and variance-covariance matrix Σ, that capture the systematic variation of the response at the subject-specific level. The residuals e_{ij} are assumed to be normally distributed with mean 0 and variance σ^2_e.

In the most parsimonious model that we considered, both the population mean and each subject's CD4+ cell slope were linear on the square-root scale. This model can be written as,

$$\sqrt{CD4}_{ij} = \alpha_o + (t_{ij} - 2)\alpha_1 + \beta_{io} + (t_{ij} - 2)\beta_{i1} + e_{ij},$$

where t_{ij} is the time (in years from seroconversion) at which the jth measurement on the ith individual was taken, α_0 and α_1 are the population mean intercept and slope, respectively, and β_{0i} and β_{1i} determine the subject-specific intercepts and slopes. Using this parameterization, the intercept term α_0 (baseline) refers to the time 2 years after seroconversion, the point beyond which there is substantial follow-up.